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09/509239

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Request for grant of a Patent

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① Title of invention

1 Please give the title of the invention: VACCINE

② Applicant's details

☐ **First or only applicant**

2a If you are applying as a corporate body please give:

Corporate Name: SmithKline Beecham Biologicals s.a.

Country *(and State of incorporation, if appropriate)*: Belgium

2b If you are applying as an individual or one of a partnership please give in full:

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2c **In all cases,** please given the following details:

Address: Rue de l'institut 89, B-1330, Rixensart

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⓫

please give details below

Agent's name MARCUS J W DALTON

Agent's address SmithKline Beecham
Corporate Intellectual Property
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Brentford, Middlesex

Postcode TW8 9EP
Agent's ADP number

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4. Agent's or applicant's reference number *(if applicable)* MJWD/B45110

⑤ Claiming an earlier application date

5. Are you claiming that this application be treated as having been filed on the date of filing of an earlier application?

Yes ☐ No ☒ *go to 6*

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❼

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❼ Inventorship

7. Are you (the applicant or applicants) the sole inventor or the joint inventors?
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Yes ☐ No ☒ ➲ *A Statement of Inventorship on Patents form 7/77 will need to be filed (see Rule 15).*

❽ *Please supply duplicates of claim(s), abstract, description and drawings).*

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8a Please fill in the number of sheets for each of the following types of document contained in this application

Continuation sheets for this Patents Form 1/77			–
Claim(s)	2	Description	19
Abstract		Drawing(s)	7

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Patents Form 10/77 - Request for Substantive Examination	–

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Signed Marcus J W Dalton Date: 26/09/97 *(day month year)*

MARCUS J W DALTON
Chartered Patent Attorney
Attorney for the Applicant

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MJWD1891/jkm

VACCINE

The present invention relates to novel HIV protein constructs, to their use in medicine, to pharmaceutical compositions containing them and to methods of their manufacture.

In particular, the invention relates to fusion proteins comprising HIV-1 NEF and/or TAT proteins.

HIV-1 is the primary cause of the acquired immune deficiency syndrome (AIDS) which is regarded as one of the world's major health problems. Although extensive research throughout the world, has been conducted to produce a vaccine, such efforts thus far, have not been successful.

Non-envelope proteins of HIV-1 have been described and include for example internal structural proteins such as the products of the *gag* and *pol* genes and, other non-structural proteins such as Rev, Nef, Vif and Tat (Greene et al., New England J. Med, **324**, 5, 308 et seq (1991) and Bryant et al. (Ed. Pizzo), Pediatr. Infect. Dis. J., **11**, 5, 390 et seq (1992).

HIV NEF and TAT proteins are early proteins, that is they are expressed early in infection and in the absence of structural proteins.

According to the present invention there is provided a NEF or TAT protein derived from Pichia Pastoris. In one embodiment the NEF or TAT proteins are linked to an immunological fusion protein.

Preferably the fusion protein is protein D or it lipidated derivative Lipoprotein D, from Haemophilius influenzae B. In particular, it is preferred that the N-terminal third, i.e. approximately the first 100-130 amino acids are utilised. Alternatively and in a preferred embodiment the NEF protein may be linked to the TAT proteins. Such NEF-TAT fusions may optionally also be linked to an immunological fusion partner, such as protein D.

The proteins of the present invention preferably contain a C terminial Histidine tail which comprises between 5-10 Histidine residues. This aids purification . In an embodiment of the invention, NEF-TAT fusion proteins are optionally linked to a non HIV immunological fusion partner. Preferred constructs

Enzymatic polymerisation of DNA may be carried out *in vitro* using a DNA polymerase such as DNA polymerase I (Klenow fragment) in an appropriate buffer containing the nucleoside triphosphates dATP, dCTP, dGTP and dTTP as required at a temperature of 10°-37°C, generally in a volume of 50μl or less. Enzymatic ligation of DNA fragments may be carried out using a DNA ligase such as T4 DNA ligase in an appropriate buffer, such as 0.05M Tris (pH 7.4), 0.01M $MgCl_2$, 0.01M dithiothreitol, 1mM spermidine, 1mM ATP and 0.1mg/ml bovine serum albumin, at a temperature of 4°C to ambient, generally in a volume of 50ml or less. The chemical synthesis of the DNA polymer or fragments may be carried out by conventional phosphotriester, phosphite or phosphoramidite chemistry, using solid phase techniques such as those described in 'Chemical and Enzymatic Synthesis of Gene Fragments - A Laboratory Manual' (ed. H.G. Gassen and A. Lang), Verlag Chemie, Weinheim (1982), or in other scientific publications, for example M.J. Gait, H.W.D. Matthes, M. Singh, B.S. Sproat, and R.C. Titmas, Nucleic Acids Research, 1982, 10, 6243; B.S. Sproat, and W. Bannwarth, Tetrahedron Letters, 1983, 24, 5771; M.D. Matteucci and M.H. Caruthers, Tetrahedron Letters, 1980, 21, 719; M.D. Matteucci and M.H. Caruthers, Journal of the American Chemical Society, 1981, 103, 3185; S.P. Adams *et al.*, Journal of the American Chemical Society, 1983, 105, 661; N.D. Sinha, J. Biernat, J. McMannus, and H. Koester, Nucleic Acids Research, 1984, 12, 4539; and H.W.D. Matthes *et al.*, EMBO Journal, 1984, 3, 801.

The process of the invention may be performed by conventional recombinant techniques such as described in Maniatis *et al.*, Molecular Cloning - A Laboratory Manual; Cold Spring Harbor, 1982-1989.

In particular, the process may comprise the steps of :

i) preparing a replicable or integrating expression vector capable, in a host cell, of expressing a DNA polymer comprising a nucleotide sequence that encodes the protein or an immunogenic derivative thereof

ii) transforming a host cell with said vector

described in, for example, Maniatis *et al.* cited above, or "DNA Cloning" Vol. II, D.M. Glover ed., IRL Press Ltd, 1985.

The choice of transforming conditions is determined by the host cell. Thus, a bacterial host such as E. coli may be treated with a solution of $CaCl_2$ (Cohen *et al.*, Proc. Nat. Acad. Sci., 1973, *69*, 2110) or with a solution comprising a mixture of RbC1, $MnCl_2$, potassium acetate and glycerol, and then with 3-[N-morpholino]-propane-sulphonic acid, RbC1 and glycerol. Mammalian cells in culture may be transformed by calcium co-precipitation of the vector DNA onto the cells. The invention also extends to a host cell transformed with a replicable expression vector of the invention.

Culturing the transformed host cell under conditions permitting expression of the DNA polymer is carried out conventionally, as described in, for example, Maniatis *et al.* and "DNA Cloning" cited above. Thus, preferably the cell is supplied with nutrient and cultured at a temperature below 50°C.

The product is recovered by conventional methods according to the host cell. Thus, where the host cell is bacterial, such as E. coli - or yeast such as Pichia; it may be lysed physically, chemically or enzymatically and the protein product isolated from the resulting lysate. Where the host cell is mammalian, the product may generally be isolated from the nutrient medium or from cell free extracts. Conventional protein isolation techniques include selective precipitation, adsorption chromatography, and affinity chromatography including a monoclonal antibody affinity column.

As the proteins of the present invention are provided with Histidine tails, purification can easily be achieved by the use of a metal ion attinity column. In a preferred embodiment, the protein is further purified by subjecting it to cation ion exchange chromatography and/or Gel filtration chromatography. The protein is then sterilised by passing through a 0.22 μm membrane.

The proteins of the invention can then be formulated as a vaccine, or the Histidine residues enzymatically cleared.

Preferably the formulation additional comprises an oil in water emulsion and tocopherol. The present invention also provides a method for producing a vaccine formulation comprising mixing a protein of the present invention together with a pharmaceutically acceptable excipient, such as 3D-MPL.

The vaccine of the present invention may additional comprise further HIV proteins, such as the envelope glycoprotein gp160 or its derivative gp 120.

The invention will be further described by reference to the following examples:

EXAMPLES:

General

Nef and Tat proteins, two regulatory proteins encoded by the human immunodeficiency virus (HIV-1) were produced in *E.coli* and in the methylotrophic yeast *Pichia pastoris*.

The Nef gene from the Bru/Lai isolate (Cell 40: 9-17, 1985) was selected for these constructs since this gene is among those that are most closely related to the consensus Nef .

The starting material for the Bru/Lai Nef gene was a 1170bp DNA fragment cloned on the mammalian expression vector pcDNA3 (pcDNA3/Nef).

The Tat gene originates from the BH10 molecular clone. This gene was received as an HTLV III cDNA clone named pCV1 and described in Science, 229, p69-73, 1985.

1. EXPRESSION OF HIV-1 NEF AND TAT SEQUENCES IN E.COLI.

Sequences encoding the Nef protein as well as a fusion of Nef and Tat sequences were placed in plasmids vectors: pRIT14586 and pRIT14589 (see figure 1).

NcoI

PRIMER 01: 5'ATCGTCCATG.GGT.GGC.AAG.TGG.T 3'

SpeI

PRIMER 02: 5' CGGCTACTAGTGCAGTTCTTGAA 3'

The Nef DNA region amplified starts at nucleotide 8357 and terminates at nucleotide 8971 (Cell, 40: 9-17, 1985).

An NcoI restriction site (with carries the ATG codon of the Nef gene) was introduced at the 5'end of the PCR fragment while a SpeI site was introduced at the 3' end.

The PCR fragment obtained and the expression plasmid pRIT14586 were both restricted by NcoI and SpeI, purified on an agarose gel, ligated and transformed in the appropriate *E.coli* host cell, strain AR58.This strain is a cryptic λ lysogen derived from N99 that is *gal*E::Tn10, Δ-8 (*chl*D-*pgl*), Δ-H1 (*cro-chl*A), N^+, and cI857.

The resulting recombinant plasmid received, after verification of the Nef amplified region by automatic sequencing,(see section 1.1.2 below) the pRIT14595 denomination.

1.1.2 Selection of transformants of *E. Coli* strain AR58 with pRIT14595

When transformed in AR58 *E.coli* host strain, the recombinant plasmid directs the heat-inducible production of the heterologous protein.

Heat inducible protein production of several recombinant lipoD-Nef-His transformants was analysed by Coomassie Blue stained SDS-PAGE. All the transformants analysed showed an heat inducible heterologous protein production.

The abundance of the recombinant Lipo D-Nef-Tat-His fusion protein was estimated at 10% of total protein.

1.3 CONSTRUCTION OF RECOMBINANT STRAIN ECLD-NT6 PRODUCING THE LIPOD-NEF-TAT-HIS FUSION PROTEIN.

1.3.1 Construction of the lipoD-Nef-Tat-His expression plasmid pRIT14596

The Tat gene(BH10 isolate) was amplified by PCR from a derivative of the pCV1 plasmid with primers 03 and 04. SpeI restriction sites were introduced at both ends of the PCR fragment.

SpeI

PRIMER 03: 5' ATCGTACTAGT.GAG.CCA.GTA.GAT.C 3'

SpeI

PRIMER 04: 5' CGGCTACTAGTTTCCTTCGGGCCT 3'

The nucleotide sequence of the amplified Tat gene is illustrated in the pCV1 clone (Science 229 : 69-73, 1985) and covers nucleotide 5414 till nucleotide 7998. The PCR fragment obtained and the plasmid pRIT14595 (expressing lipoD-Nef-His protein) were both digested by SpeI restriction enzyme, purified on an agarose gel, ligated and transformed in competent AR58 cells. The resulting recombinant plasmid received, after verification of the Tat amplified sequence by automatic sequencing (see section 1.3.2 below), the pRIT14596 denomination.

1.3.2 Selection of transformants of strain AR58 with pRIT14596

Transformants were grown, heat induced and their proteins were analysed by Coomassie Blue stained gels. The production level of the recombinant protein was estimated at 1% of total protein. One recombinant strain was selected and received the laboratory denomination ECLD-NT6.

To express these HIV-1 genes a modified version of the integrative vector PHIL-D2 (INVITROGEN) was used. This vector was modified in such a way that expression of heterologous protein starts immediately after the native ATG codon of the AOX1 gene and will produce recombinant protein with a tail of one glycine and six histidines residues . This PHIL-D2-MOD vector was constructed by cloning an oligonucleotide linker between the adjacent AsuII and EcoRI sites of PHIL-D2 vector. In addition to the His tail, this linker carries NcoI, SpeI and XbaI restriction sites between which Nef, Tat and Nef-Tat fusion were inserted.

2.1 **CONSTRUCTION OF THE INTEGRATIVE VECTORS pRIT14597** (encoding Nef-His protein), pRIT14598 (encoding Tat-His protein) and pRIT14599 (encoding fusion Nef-Tat-His).

The Nef gene was amplified by PCR from the pcDNA3/Nef plasmid with primers 01 and 02(see section 1.1.1 construction of pRIT14595).The PCR fragment obtained and the integrative PHIL-D2-MOD vector were both restricted by NcoI and SpeI, purified on agarose gel and ligated to create the integrative plasmid pRIT14597.

The Tat gene was amplified by PCR from a derivative of the pCV1 plasmid with primers 05 and 04(see section 1.3.1 construction of pRIT14596):

NcoI

PRIMER 05 5'ATCGTCCATGGAGCCAGTAGATC 3'

An NcoI restriction site was introduced at the 5' end of the PCR fragment while a SpeI site was introduced at the 3' end with primer 04. The PCR fragment obtained and the PHIL-D2-MOD vector were both restricted by NcoI and SpeI, purified on agarose gel and ligated to create the integrative plasmid pRIT14598.

To construct pRIT14599, a 910bp DNA fragment corresponding to the Nef-Tat-His coding sequence was ligated between the EcoRI blunted(T4 polymerase)

°A threonine and a serine introduced by cloning procedure

°One glycine and six histidines

Strain Y1737(Mut^S phenotype) producing the recombinant Nef-Tat-His fusion protein, a myristylated 302 amino acids protein which is composed of:

°Myristic acid

°A methionine, created by the use of NcoI cloning site

°205a.a. of Nef protein(starting at a.a.2 and extending to a.a.206)

°A threonine and a serine created by the cloning procedure

°85a.a. of the Tat protein(starting at a.a.2 and extending to a.a.86)

°A threonine and a serine introduced by the cloning procedure

°One glycine and six histidines

Carboxymethylation (1/2 h - room temperature - in the dark)	+ 0,25M Iodoacetamid (powder addition) / pH adjusted to 7.5 (with 0,5M NaOH solution) befor incubation
↓	
Immobilized metal ion affinity chromatography on Ni^{++}-NTA-Agarose (Qiagen - 30 ml of resin)	Equilibration buffer: 10 mM PO_4 pH 7.5 - 150m NaCl - 4.0M GuHCl Washing buffer: 1) Equilibration buffer 2) 10 mM PO_4 pH 7.5 - 150mM NaCl - 6M Urea 3) 10 mM PO_4 pH 7.5 - 150mM NaCl - 6M Urea - 25 mM Imidazol Elution buffer: 10 mM PO_4 pH 7.5 - 150mM Na - 6M Urea - 0,5M Imidazol
↓	
Dilution	Down to an ionic strength of 18 mS/cm² Dilution buffer: 10 mM PO_4 pH 7.5 - 6M Urea
↓	
Cation exchange chromatography on SP Sepharose FF (Pharmacia - 30 ml of resin)	Equilibration buffer: 10 mM PO_4 pH 7.5 - 150mM NaCl - 6.0M Urea Washing buffer: 1) Equilibration buffer 2) 10 mM PO_4 pH 7.5 - 250m NaCl - 6M Urea Elution buffer: 10 mM Borate pH 9.0 - 2M NaCl 6M Urea -
↓	
Concentration	up to 5 mg/ml 10kDa Omega membrane(Filtron)
↓	
Gel filtration chromatography on Superdex200 XK 16/60 (Pharmacia - 120 ml of resin)	Elution buffer: 10 mM PO_4 pH 7.5 - 150mM Na - 6M Urea
↓	
Dialysis (O/N - 4°C)	Buffer: 10 mM PO_4 pH 6.8 - 150mM NaCl - Arginin*

Experiments performed at Smith Kline Beecham Biologicals have demonstrated a clear synergistic effect of combinations of MPL and QS21 in the induction of both humoral and TH1 type cellular immune responses.

The oil/water emulsion is composed of 2 oils (a tocopherol and squalene), and of PBS containing Tween 80 as emulsifier. The emulsion comprised 5% squalene 5% tocopherol 0.4% Tween 80 and had an average particle size of 180 nm and is known as SB62 (see WO 95/17210).
Experiments performed at Smith Kline Beecham Biologicals have proven that the adjunction of this O/W emulsion to MPL/QS21 further increases their immunostimulant properties.

Preparation of emulsion SB62 (2 fold concentrate)

Tween 80 is dissolved in phosphate buffered saline (PBS) to give a 2% solution in the PBS. To provide 100 ml two fold concentrate emulsion 5g of DL alpha tocopherol and 5ml of squalene are vortexed to mix thoroughly. 90ml of PBS/Tween solution is added and mixed thoroughly. The resulting emulsion is then passed through a syringe and finally microfluidised by using an M110S microfluidics machine. The resulting oil droplets have a size of approximately 180 nm.

Preparation of oil in water formulation.

Antigen prepared in accordance with example 1 or 2 (5μg) was diluted in 10 fold concentrated PBS pH 6.8 and H_2O before consecutive addition of SB62, 3 D MPL (5μg), QS21 (5μg) and 50 μg/ml thiomersal as preservative at 5 min interval. The emulsion volume is equal to 50% of the total volume (50μl for a dose of 100μl).

All incubations were carried out at room temperature with agitation.

* **FIGURE 2**

◊ **Pichia-expressed constructs (plain constructs)**

⇒ *NEF - HIS*

DNA sequence

ATGGGTGGCAAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGA
ATGAGACGAGCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAA
AAACATGGAGCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGG
CTAGAAGCACAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTA
AGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGGGG
GGACTGGAAGGGCTAATTCACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATC
TACCACACACAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTC
AGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAG
GTAGAAGAGGCCAATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCAT
GGAATGGATGACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCA
TTTCATCACGTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGGC
CACCATCACCATCACCATTAA

Protein sequence

MGGKWSKSSVVGWPTVRERMRRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAW
LEAQEEEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDLWI
YHTQGYFPDWQNYTPGPGVRYPLTFGWCYKLVPVEPDKVEEANKGENTSLLHPVSLH
GMDDPEREVLEWRFDSRLAFHHVARELHPEYFKNCTSGHHHHHH.

⇒ *TAT - HIS*

DNA sequence

ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAA
ACTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCATTGCCAAGTTTGTTTC
ATAACAAAAGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGA
CCTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAA
TCCCGAGGGGACCCGACAGGCCCGAAGGAAACTAGTGGCCACCATCACCATCACCAT
TAA

Protein sequence

MEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITKALGISYGRKKRRQRRR
PPQGSQTHQVSLSKQPTSQSRGDPTGPKETSGHHHHHH.

*

ATGGATCCAAAAACTTTAGCCCTTTCTTTATTAGCAGCTGGCGTACTAGCAGGTTGT
AGCAGCCATTCATCAAATATGGCGAATACCCAAATGAAATCAGACAAAATCATTATT
GCTCACCGTGGTGCTAGCGGTTATTTACCAGAGCATACGTTAGAATCTAAAGCACTT
GCTTTTGCACAACAGGCTGATTATTTAGAGCAAGATTTAGCAATGACTAAGGATGGT
CGTTTAGTGGTTATTCACGATCACTTTTTAGATGGCTTGACTGATGTTGCGAAAAAA
TTCCCACATCGTCATCGTAAAGATGGCCGTTACTATGTCATCGACTTTACCTTAAAA
GAAATTCAAAGTTTAGAAATGACAGAAAACTTTGAAACCATGGGTGGCAAGTGGTCA
AAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGAGCTGAGCCA
GCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGAGCAATCACA
AGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGGCTAGAAGCACAAGAGGAG
GAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTACAAG
GCAGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGGGGGGACTGGAAGGGCTAATT
CACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTAC
TTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTCAGATATCCACTGACCTTT
GGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAA
GGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGAATGGATGACCCTGAG
AGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTCATCACGTGGCCCGA
GAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGGCCACCATCACCATCACCAT
TAA

Protein sequence of the processed lipidated ProtD-NEF-HIS protein

(Amino-acids corresponding to Prot D fusion partner are in bold)

CSSHSSNMANTQMKSDKIIIAHRGASGYLPEHTLESKALAFAQQADYLEQDLAMTKD
GRLVVIHDHFLDGLTDVAKKFPHRHRKDGRYYVIDFTLKEIQSLEMTENFETMGGKW
SKSSVVGWPTVRERMRRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAWLEAQE
EEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDLWIYHTQG
YFPDWQNYTPGPGVRYPLTFGWCYKLVPVEPDKVEEANKGENTSLLHPVSLHGMDDP
EREVLEWRFDSRLAFHHVARELHPEYFKNCTSGHHHHHH.

⇒ ***LipoD-NEF-TAT-HIS***

DNA sequence

Nucleotides corresponding to the Prot D Fusion Partner are in bold.
The Lipidation Signal Sequence is underlined. After processing, the cysteine coded by the TGT codon, indicated with a star, becomes the amino terminal residue which is then modified by covalently bound fatty acids.

ATGGATCCAAGCAGCCATTCATCAAATATGGCGAATACCCAAATGAAATCAGACAAA
ATCATTATTGCTCACCGTGGTGCTAGCGGTTATTTACCAGAGCATACGTTAGAATCT
AAAGCACTTGCGTTTGCACAACAGGCTGATTATTTAGAGCAAGATTTAGCAATGACT
AAGGATGGTCGTTTAGTGGTTATTCACGATCACTTTTTAGATGGCTTGACTGATGTT
GCGAAAAAATTCCCACATCGTCATCGTAAAGATGGCCGTTACTATGTCATCGACTTT
ACCTTAAAAGAAATTCAAAGTTTAGAAATGACAGAAAACTTTGAAACCATGGGTGGC
AAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGA
GCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGA
GCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGGCTAGAAGCA
CAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATG
ACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGGGGGGACTGGAA
GGGCTAATTCACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACA
CAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTCAGATATCCA
CTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAG
GCCAATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGAATGGAT
GACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTCATCAC
GTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGGCCACCATCAC
CATCACCATTAA

Protein sequence

(Amino-acids corresponding to Prot D fusion partner are in bold)

MDPSSHSSNMANTQMKSDKIIIAHRGASGYLPEHTLESKALAFAQQADYLEQDLAMT
KDGRLVVIHDHFLDGLTDVAKKFPHRHRKDGRYYVIDFTLKEIQSLEMTENFETMGG
KWSKSSVVGWPTVRERMRRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAWLEA
QEEEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDLWIYHT
QGYFPDWQNYTPGPGVRYPLTFGWCYKLVPVEPDKVEEANKGENTSLLHPVSLHGMD
DPEREVLEWRFDSRLAFHHVARELHPEYFKNCTSGHHHHHH.

⇒ *ProtD-NEF -TAT-HIS*

DNA sequence

Nucleotides corresponding to the Prot D Fusion Partner are in bold.

ATGGATCCAAGCAGCCATTCATCAAATATGGCGAATACCCAAATGAAATCAGACAAA
ATCATTATTGCTCACCGTGGTGCTAGCGGTTATTTACCAGAGCATACGTTAGAATCT
AAAGCACTTGCGTTTGCACAACAGGCTGATTATTTAGAGCAAGATTTAGCAATGACT
AAGGATGGTCGTTTAGTGGTTATTCACGATCACTTTTTAGATGGCTTGACTGATGTT
GCGAAAAAATTCCCACATCGTCATCGTAAAGATGGCCGTTACTATGTCATCGACTTT

CLAIMS

1. An HIV NEF protein or derivative linked to an immunological fusion partner or an HIV TAT protein.

2. HIV NEF-TAT fusion protein.

3. A protein as claimed in Claim 1 wherein the immunological fusion partner is a Haemophilus Influenza B protein D or derivative thereof.

4. A Protein as claimed in Claim 1 wherein the fusion partner is a lipoprotein D or derivative thereof.

5. A protein as claimed in Claim 3 or 4 wherein the fusion partner comprises between 90-130 amino acid from the N terminal of protein D.

6. A protein as claimed in Claim 1 to 5, wherein the NEF protein is the entire NEF protein.

7. A protein as claimed in Claim 1 to 6, wherein the NEF protein is fused to an HIV TAT protein and an immunological fusion partner.

8. A protein as claimed in Claim 1 to 7, wherein the protein has a Histidine tail.

9. A nucleic acid encoding a protein of Claim 1 to 8.

10. A host transformed with a nucleic acid of Claim 9.

11. A vaccine comprising a protein of any of Claim 1 to 8 inadmixture with a pharmaceutically acceptable excipient.